



A new species of toad (Bufonidae: *Incilius*) from central Panama

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Abstract

We describe *Incilius karenlipsae* **sp. nov.**, a new species of toad known from a single locality in the Cordillera de Talamanca of central Panama. We describe this species based on a single individual of perhaps what is now an extinct species. We present mitochondrial sequence data from *cyt b* (675 base-pairs, bp) and 16S (566 bp) to infer its phylogenetic placement among other bufonids. The new species is closely related to the more widespread species *I. coniferus*, but is clearly distinguishable from it by its larger size, vestigial parotoid glands and extensively developed fleshy pads on the hands and feet.

Key words: Anura, Bufonidae, Panama, Coclé Province, new species, *Incilius karenlipsae*, *Incilius coniferus*

Resumen

Describimos *Incilius karenlipsae* **sp. nov.**, una especie de rana nueva encontrada en un lugar en la Cordillera de Talamanca de Panamá central. Describimos esta especie basado en un individuo, que potencialmente representa una especie extinta. Presentamos datos de la secuencia de ADN mitocondrial de *cyt b* (675 parejas de bases, pb) y 16S (566 pb) para inferir su posición filogenética con respecto a otros bufonidos. La especie nueva está relacionada a la especie distribuida más ampliamente *I. coniferus*, pero es claramente distinguida por su tamaño más grande, glándulas parótidas rudimentarias, y almohadillas carnosas desarrolladas extensamente en las manos y los pies.

Palabras clave: Anura, Bufonidae, Panama, Provincia Coclé, especie nueva, *Incilius karenlipsae*, *Incilius coniferus*

Introduction

In 2005, as the devastating pathogenic amphibian chytrid fungus *Batrachochytrium dendrobatidis* was expanding in a wave-like manner across central Panama (Lips *et al.*, 2006), we collected amphibians in the Cordillera de Talamanca to create captive survival assurance colonies (Gagliardo *et al.*, 2008). Our field work resulted in the discovery of a remarkable new species of hylid frog (*Ecnomiohyla rabborum*; Mendelson *et al.*, 2008) and also a new species of bufonid frog described herein. The discovery of new species just as their populations were being decimated—or even eradicated—underscores the significant lacunae in our understanding of true amphibian diversity (Gascon *et al.*, 2007) and the horrific scope of the global crisis of amphibian extinctions (Stuart *et al.*, 2004; Collins and Crump, 2009). Indeed, it is often the case that narrowly endemic species such as the two frogs discovered in 2005 are eliminated by amphibian chytridiomycosis, resulting in regional homogenization of amphibian faunas (Smith *et al.*, 2009). Herein we describe yet another potentially ill-fated species—a practice we have come to call “forensic taxonomy” that is becoming all too familiar (e.g., Coloma *et al.*, 2007) in the wake of ongoing amphibian extinctions.

Material and methods

General terminology and measurements are those of Mendelson (1997). The single specimen is identified as an adult male with a vocal slit and nuptial excrescences, characteristics normally lacking in large adult females. Foot-webbing formulae follow the system of Savage and Heyer (1967), as modified by Myers and Duellman (1982) and Savage and Heyer (1997). The general format of the description and diagnosis is formatted similar to that of Mendelson *et al.* (2005). Comparative information about *Incilius coniferus* and related species was gathered from Savage (2002), and from direct examination of specimens (Appendix I). Museum abbreviations are those proposed by Leviton *et al.* (1985).

We collected sequence data from the single specimen (UTA A-59522) representing two mitochondrial genes (16S and cyt b) using the primers listed in Mulcahy and Mendelson (2000); DNA extraction, PCR protocols, and sequence reactions followed Mendelson *et al.* (*submitted*). To infer the phylogenetic position of this species, we aligned sequences with those from all other species of bufonids in the genus *Incilius* for which sequence data were available (Mendelson *et al.*, *submitted*). We used parsimony and Bayesian analyses using PAUP* (v4.0b10; Swofford 2000) and MrBayes (Ronquist and Huelsenbeck, 2003), respectively. Parsimony analyses used heuristic search options with 100 random, step-wise additions, tree bisection-reconnection branch swapping algorithm, and saving multiple best-trees. Bootstrap analyses used 100 replicates, with 100 random-additions per replicate. Bayesian analyses were done with the data separated into four partitions, one for each codon position cyt b and one for the RNA 16S. Each partition was analyzed in MrModeltest v2.2 (Nylander, 2004) to determine the best model of nucleotide substitution under the Akaike Information Criteria. Two analyses were run for 10 million generations each, saving trees every 1000, with four heated chains (user defaults). Stationarity was assessed by visual plots in Tracer and the first 2500 trees (of 10,000) were discarded as the burnin. A 50% majority consensus was taken from the remaining trees and posterior probabilities of 0.95 or above were considered significant.

We collected 566 base-pairs (bp) of 16S and 675 bp of cyt b from UTA A-59522 [GenBank Accession Nos. GU552454 (16S) & GU552455 (cyt b)], and when placed in the alignment of 16S and cyt b for other *Incilius* species (Mendelson *et al.*, *submitted*) resulted in a total of 1244 bp, with 375 parsimony-informative characters, 799 were constant. Parsimony analyses resulted in 19 trees of 2069 steps, and a strict consensus with bootstrap support values of 100% places the new species as sister to *I. coniferus*, from which it differed by 2.8% of uncorrected pair-wise sequence divergence. Bayesian analyses reached convergence with average standard deviation of split frequencies = 0.004, with an average -lnl = 10257.33, and placed UTA A-59522 sister to *I. coniferus* with posterior probability = 1.0. The respective phylogenies (parsimony and Bayesian) differed only by the inclusion of this taxon with Mendelson *et al.* (*submitted*) as sister to *I. coniferus* (i. e. the inclusion this specimen did not change the relationships among other species of *Incilius*). Based on these differences and a distinctive morphology we describe the toad as a new species of *Incilius*.

Incilius karenlipsae Mendelson & Mulcahy, new species

Figs. 1–2

Holotype. UTA A-59522 (original number JRM 4965), an adult male from Panama: Coclé Province: El Copé, Parque Nacional G. D. Omar Torrijos (8° 40' N, 80° 37' 17" W; approximately 850m), obtained by Mason J. Ryan on 15 June 2005.

Diagnosis. The new species is allocated to the genus *Incilius* (sensu Frost *et al.*, 2009) by the presence of a full complement of cranial crests and the presence (if indistinct) of a lateral descending row of enlarged tubercles on the body. The species is allocated to this genus additionally on the basis of a phylogenetic analysis of mitochondrial DNA sequence-data; that analysis included nearly every known species of *Incilius* (discussed below). A large species of *Incilius* (single known male 89.3 mm SVL; females unknown), having the following combination of characters: (1) tympanum evident, distinctly vertically ovoid, similar in size of the orbit; (2) canthal, supraorbital, supratympanic, postorbital, parietal preorbital, pretympanic, supralabial

crests present; (3) cranial crests moderately developed, low, thin; (4) tibia short, about 36% SVL; (5) feet short, about 35% SVL; (6) dorsal tubercles very small, pointed; (7) ventral skin smooth; (8) lateral descending row of enlarged tubercles indistinct, marked only by an intermittent series of sharply pointed tubercles slightly larger than other dorsal tubercles; (9) vocal slit unilateral; (10) snout shape bluntly rounded in lateral view, sharply pointed in dorsal view; (11) parotoid glands vestigial, evident only as a small cluster of tiny pores visible under dissecting microscope; (12) skin between cranial crests on top of head between cranial crests smooth; (13) dorsal coloration uniformly dark brown to black, ventral coloration dull cream with scattered small black markings.

Incilius karenlipsae can be distinguished from all species of *Incilius*, and all species of *Rhinella* and *Rhaebo* occurring in Panama, by the unique absence of a developed parotoid gland and the unusual fully webbed hands and feet. In general appearance, *I. karenlipsae* is most similar to *I. coniferus* but differs additionally from that species by having less spinose skin and by being larger (males in *I. coniferus* to 72 mm SVL).

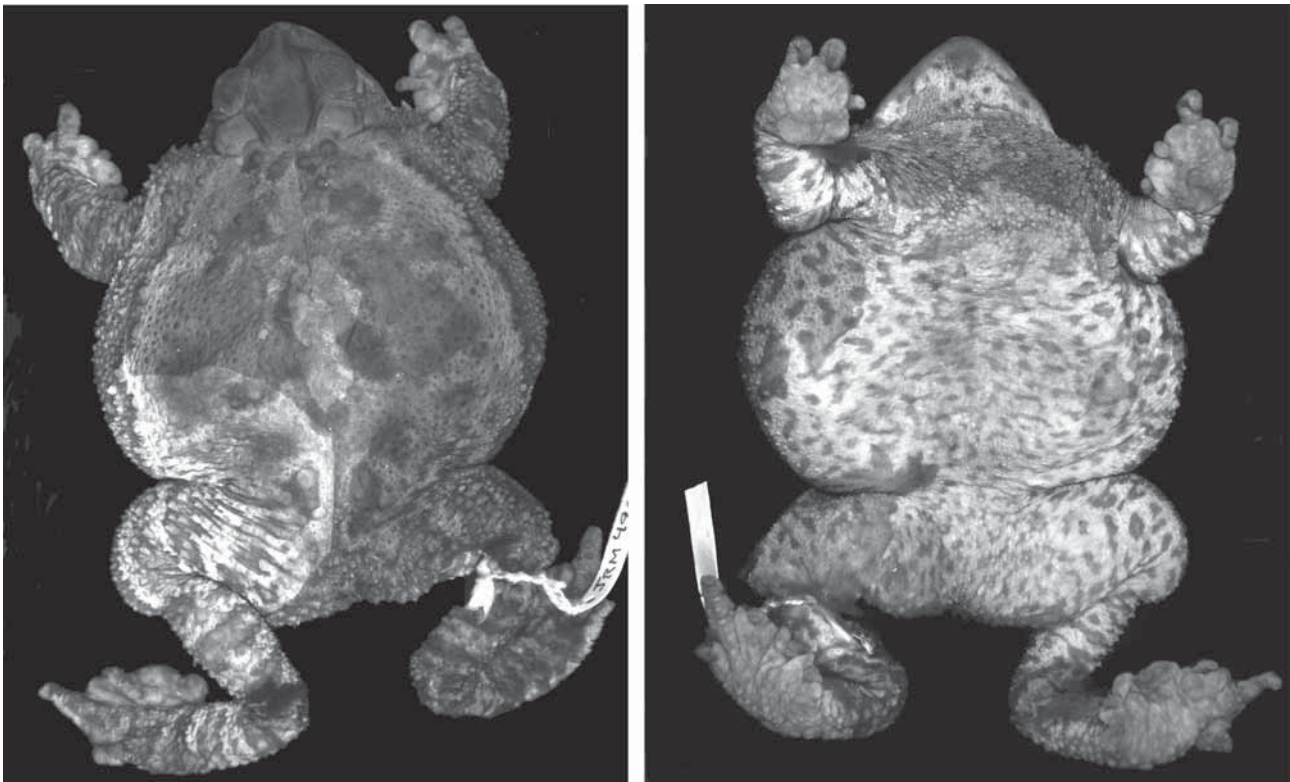


FIGURE 1. Dorsal and ventral views of the holotype and only known specimen of *Incilius karenlipsae* (UTA A-59522; adult male; SVL = 89.3 mm). Note that superficial epidermal layers have come loose in some areas on the specimen. A dorsal color pattern is evident in the specimen, but in life the toad was nearly uniform gray-black dorsal coloration, regardless of temperature or other environmental variables.

Description of the holotype. Body robust; head wider than long, width 34% SVL, length 27% SVL; snout sharply pointed in dorsal view, bluntly rounded in profile, rostral keel distinct; canthal, preorbital, supraorbital, parietal, pretympenic, supratympanic, and postorbital crests present; all cranial crests moderately developed, low, thin; skin on top of head co-ossified; nostril protuberant, directed dorsally; canthus rostralis forming distinct, raised, canthal ridge; loreal region concave; lip distinct, rounded; suborbital ridge present, distinct, extending from angle of the jaw anteriorly to level of anterior margin of orbit; notch at symphysis of upper jaw present, distinct; eye–nostril distance equal in size to diameter of orbit; tympanum distinct, vertically ovoid; tympanic annulus distinct only along anterior and ventral margins, upper margin contacting supratympanic crest, posterior margin obscured by overlying skin. Forelimbs short, robust; hand broad, with short, plump fingers; relative length of fingers: $I < II < IV < III$; extensive fleshy webbing between Fingers

I–III, nearly obscuring Fingers I and II; tips of fingers not expanded, smooth dorsally, demarcated proximally by distinct dermal fold; palmar, pollical, subarticular, and supernumerary tubercles not evident, obscured by fleshy nature of ventral surface of hand; nuptial excrescences present as discrete, brown granular patch covering most of dorsal surface of Finger I. Hind limbs relatively short, slender, tibia length 36% SVL; foot length 25% SVL; tarsal fold absent; outer metatarsal tubercle large, ovoid, situated on lateral surface of foot, rather than more typically on ventral surface of the foot; inner metatarsal tubercle not evident; toes short, plump, Toes I–III not distinct from intermediate webbing; relative length of toes: $I < II < III < V < IV$; webbing very thick, webbing formula $I0-0II0-1III0-2IV3-0V$; tips of toes not expanded, smooth dorsally, tips of Toes IV–V demarcated distally by distinct dermal fold on dorsal surface; subarticular and supernumerary tubercles absent, being obscured by fleshy ventral surface of foot.

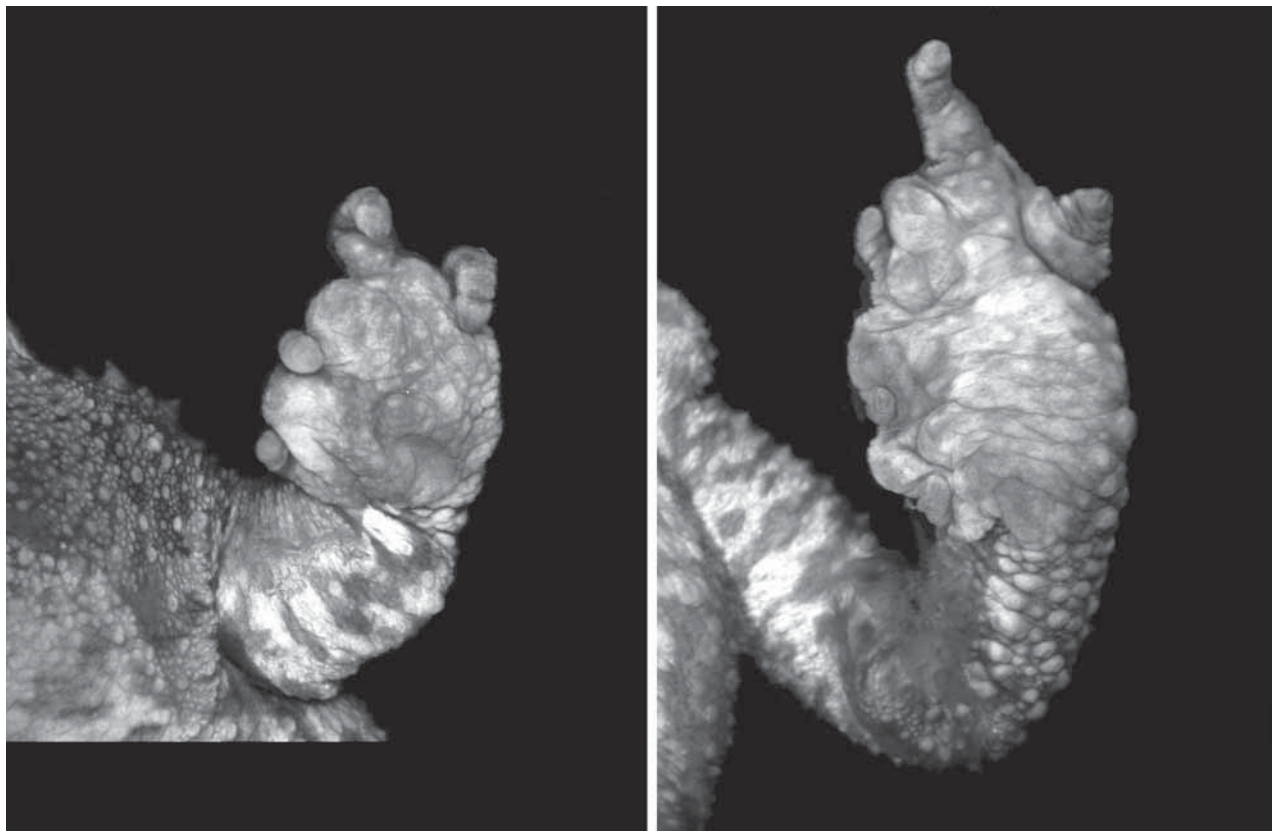


FIGURE 2. Hand and foot of the holotype of *Incilius karenlipsae* (UTA A-59522; adult male; SVL = 89.3 mm). Note that fingers on the hand are unnaturally flexed ventrally in the preserved specimen, making evaluation of their condition difficult unless the specimen is at hand.

Skin on dorsum of body smooth with many small pointed tubercles, becoming more concentrated laterally, with scattered sharply pointed tubercles, few of which are larger than others; parotoid glands not distinct, present only as a cluster of tiny pores visible under dissecting scope; lateral descending row of enlarged tubercles indistinct, marked only by an intermittent series of sharply pointed tubercles slightly larger than other dorsal tubercles; dorsal surface of head smooth between cranial crests; dorsal surfaces of limbs covered with densely arranged sharply pointed tubercles of various sizes; skin on throat granular, other ventral surfaces smooth.

Choanae large, transversely elliptical, widely spaced; teeth and odontoids absent; tongue long, about $\frac{1}{3}$ broader posteriorly, posterior fourth of length free; vocal slit small, unilateral, sinistral.

Coloration of holotype.—In preservative (in 70% ethanol), dorsum of head, body, and limbs uniformly brown, with few scattered indistinct darker brown markings; indistinct darker brown bars present on distal sections of arms, lower legs, and feet; throat dull cream with scattered small black markings; skin over vocal sac black; remaining ventral surfaces dull cream with scattered small black markings.

The specimen was preserved after its natural death, so some superficial skin has been lost; this gives the appearance of some areas of paler coloration on the specimen that were not evident in life. We note also that the digits on the specimen are preserved in a recurved position (see Fig. 2), making evaluation of their condition difficult unless the specimen is at hand.

Measurements of the holotype (in mm). SVL 89.3, head length 23.6, head width 30.4, tibia length 32.9, foot length 29.7, orbit diameter 8.8, tympanum diameter (vertical) 5.7, supratympanic crest length 4.7.

Coloration in life. In life, the color of the specimen was rather similar to that in preservative, however the individual nearly always appeared either nearly uniform dark brown or nearly black. Slight metachrosis (i.e., lightening of the overall hue of the dorsal surfaces to a dull brown) was observed rarely and did not seem to correlate with temperature or the day-night cycle. The iris was uniform bright green.

Etymology. This distinctive new species is named in honor of Dr. Karen R. Lips who has dedicated the majority of her career to the careful scientific documentation of amphibian declines and their causes. Her contributions to enlightening us on the realities and root causes of these catastrophic losses are extraordinary. We note especially that the type locality for *Incilius karenlipsae* is El Copé, Panama, where she completed her landmark study (Lips *et al.*, 2006) that directly demonstrated the cause-and-effect relationship between the arrival of a non-native pathogen and the subsequent decimation of an amphibian fauna.

Distribution and ecology. This species is known only from the type locality, in Tropical Moist Forest. The holotype was found in a small seep crossing a trail in the forest. A description of the locality was presented by Ryan *et al.* (2008).

This toad was maintained alive for several years at Zoo Atlanta in an enclosure with a sexual pair of *I. coniferus* that regularly displayed reproductive behaviors (e.g., advertisement calls, amplexus, oviposition). While in captivity, the male holotype was often observed climbing about on branches in its enclosure—a behavior well documented in the related species *I. coniferus*. No advertisement call was ever heard while the male was maintained alive, although a typical bufonid release call was noted when the animal was grasped. No cross-species reproductive interactions of any sort were observed between the pair of *I. coniferus* and *I. karenlipsae*.

Tadpoles. The tadpole of *I. karenlipsae* is unknown; we presume that the life history of this species includes a free-living larval stage (see Remarks, below).

Remarks. Our assessment of the phylogenetic position of *I. karenlipsae*, based on the addition of this taxon to the complete analysis of Mesoamerican toads (Mendelson *et al.*, *submitted*) and indicates that this species is sister to the widespread species *I. coniferus*. Despite the considerable morphological differences between these two species (see Diagnosis, above) the holotype was originally misidentified by field crews and JRM as *I. coniferus*. A phylogenetic hypothesis for the position of *I. karenlipsae* and its apparent closest relatives is presented in Fig. 3. This hypothesis allows preliminary assessment of the evolution of certain salient characteristics in this clade of toads (Fig. 3). Although axillary amplexus clearly is the plesiomorphic condition in *Incilius* (and indeed New World toads in general), the documentation of inguinal amplexus in *I. fastidiosus* (Graybeal and de Queiroz, 1992) and the lack of observations of amplexus in other species in this clade except for *I. coniferus* (axillary) presents the intriguing possibility that the unusual behavior of inguinal amplexus may be present in additional species. All species in this clade are characterized by having reduced parotoid glands, appearing vestigial in *I. karenlipsae*. Similarly, this clade is characterized by having remarkable fleshy pads on the hands and feet, and this condition is taken to the extreme in both *I. karenlipsae* and the three species referred to “*Crepidophryne*” in which the pads are so extensive that the digits are indistinct; (see Mendelson *et al.*, *submitted*, for discussion of the taxonomic status of *Crepidophryne*). The exception to this morphology of the hands and feet is in *I. coniferus*, which evidently has experienced an evolutionary reversal to a more plesiomorphic bufonid morphology of long, slender fully distinct digits without fleshy pads. Although we do not wish to speculate on the adaptive significance of the unusual morphology of the hands and feet of these species, it is perhaps noteworthy that a similar morphology is found among species of the *Rhinella veraguensis* group that have been reported to climb on rocks and vegetation (e.g., Lehr *et al.*, 2001; Chaparro *et al.*, 2007). Even though it lacks fleshy pads, *Incilius coniferus* is well known for its proclivity to climb into trees.

Fig. 3

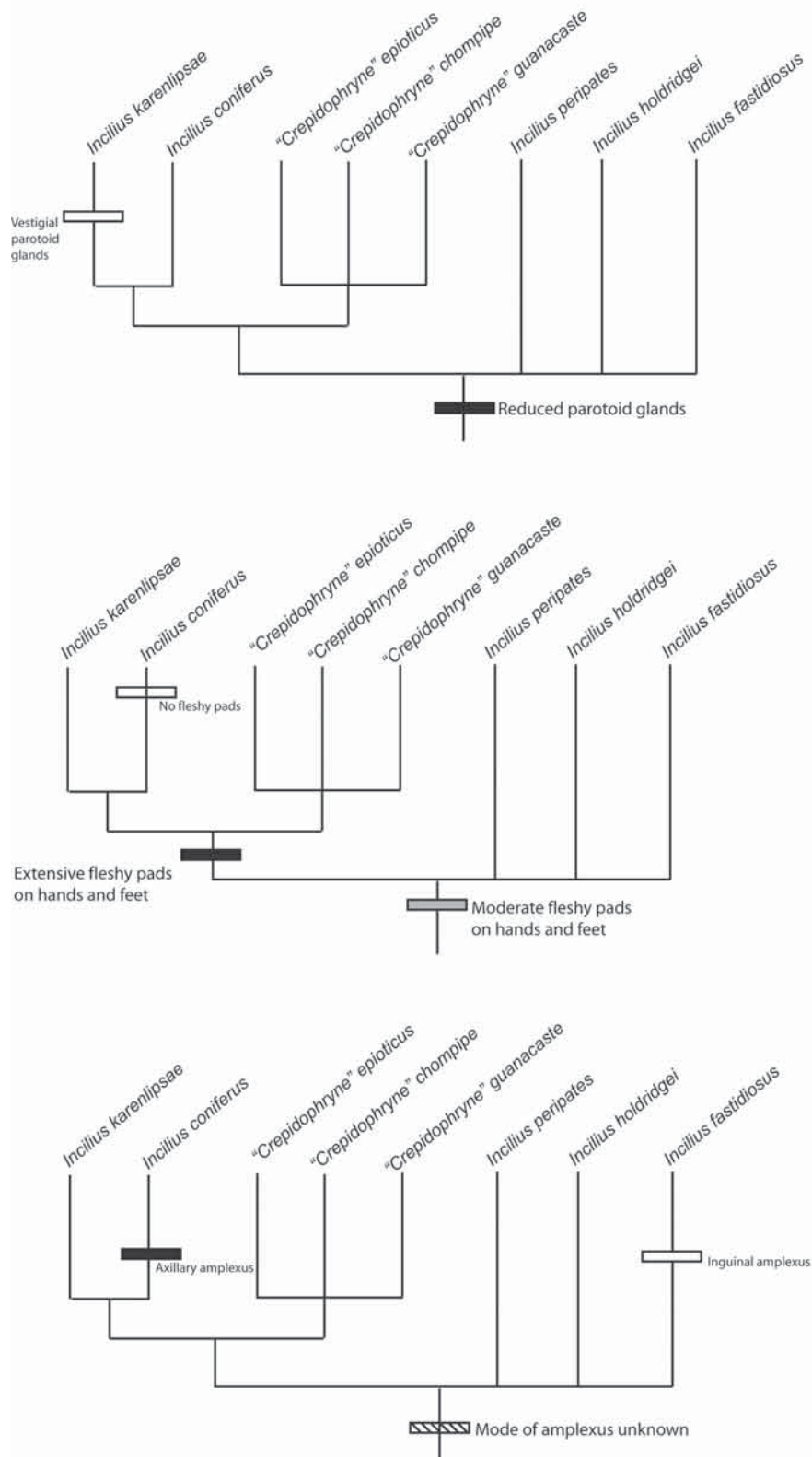


FIGURE 3. A cladogram derived from this study and Mendelson *et al.* (*submitted*) showing the phylogenetic position of *Incilius karenlipsae* as the sister taxon to *I. coniferus* determined in this study (parsimony bootstrap = 100%; Bayesian posterior-probabilities = 1.0). The phylogenetic placements of *I. peripatetes*, *I. holdridgei*, *C. epiotica*, and *C. guanacaste* are inferred based on morphology (DNA samples are not available). The evolution of several salient characteristics of the group are reconstructed; see text for discussion.

The conservation status of this species is uncertain. We are unaware of any additional specimens of this species present preserved or alive in collections in the USA or in Panama (R. Ibáñez, personal communication). We note also that this species evidently was not observed in the environs of El Copé, Panama, during extensive field surveys (summarized in Lips *et al.*, 2006). To us, this suggests that the species is relatively rare and/or secretive. Although its total distribution cannot be posited based on a single specimen, it is possible that the species is restricted to the immediate environs of Parque Nacional G. D. Omar Torrijos, El Copé, Coclé Province, Panama. The susceptibility of this species to the amphibian disease chytridiomycosis—which is now established in that region (Lips *et al.*, 2006)—is entirely unknown. Based on its presumed rarity, unknown distribution, and potential susceptibility to chytridiomycosis, we recommend that this species be recognized as Critically Endangered on the IUCN Red List (www.iucnredlist.org). We encourage additional field work in the area to determine if a population is extant.

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Appendix 1. Specimens examined

Incilius coniferus:

- Colombia: Choco: Quebrade Docordo, ca 10 km above junction with Rio San Juan (AMNH 86850); Quebrada Pangala, lower Rio San Juan, ca 17 km airline NE Palestina (AMNH 110729–33).
- Costa Rica: Limon: 50 mi NW of Limon, east riverbank, ca. 2 mi S mouth of Rio Tortuguero (AMNH 69042–43); Caño Palmas, 2–3 mi NW of Green Turtle Camp, Tortuguero (AMNH 75061–66); ca. 2 mi S Tortuguero village (AMNH 80123–24, 80137).
- Ecuador: NW Ecuador, Pambelar (AMNH 10711–12).
- Nicaragua: Kanawa (AMNH 8194); Zelaya: Maselina Creek (AMNH 8195); Cupitna Camp (AMNH 8196).
- Panama: Bocas del Toro: Peninsula Valiente, Punta Alegre (USNM 338601); Almirante (USNM 166864, 339734); Laguna de Tierra Oscura, 3.7 km S of Tiger Key (USNM 348278, 348281–84); Rio Changuinola, near Quebrada El Guabo, 16 km airline W Almirante (AMNH 107132–38); 7.5 km airline WSW Chiriquí Grande (AMNH 113883); South slope Quebrada de Arena, upper Rio Chiriquí drainage (AMNH 114555, 124081–82). Canal Zone: ca 20 km SW Colón, near Achiote (AMNH 98280–89). Chiriquí: Los Planes 23.3 km [by road] N or, on Gualaca–Chiriqui road, continental divide (USNM 339790; upper Rio Chiriquí, Fortuna Dam site (AMNH 94829–39). Coclé: El Copé, Parque Nacional Omar Torrijos (USNM 572086–90, 572092); Continental Divide N El Copé (AMNH 98290). Darien: mouth Rio Membrills [Rio Chucunaque] (AMNH 40549); Rio Chucunaque (AMNH 40620, 40635, 40653–83, 40722–23, 40730–32); above Tupisa, Rio Chucunaque (AMNH 41042). Panama: near Boqueron, Candelaria & Peluca stations (AMNH 53694–98); km 12.8 on El Llano–Carti road (AMNH 89479); Cerro Azul region, Gorgas Memorial Lab, “Sloth Camp” (AMNH 108287). Veraguas: 5–6 mi NW via road Santa Fe (AMNH 162521–27); Santa Fe, Altos de Piedra road (USNM 572091).

Incilius karenlipsae:

- Panama: Coclé: Parque Nacional G. D. Omar Torrijos (UTA A-59522).